

Overview of Heart Biomarkers

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Disclosure

- **Over the past 2 years have received research funding, honorarium for speaking and/or consultation fees regarding cardiac biomarkers from:**
 - **Abbott, Bayer, Beckman, Biopure, Biosite, Dade, i-STAT, MKI, Ortho, Roche, Response, Sensera**

“Mere mortals may not fully appreciate the complexity”..

Novel Risk Markers & Clinical Practice

- **What should the clinician look for in reports to determine implications for patient care**
 - **measure (biomarker) should add independent information about the risk or prognosis**
 - **measure should account for a large proportion of the risk associated with disease**
 - **measure should be reproducible in multiple settings**
 - **if measure is to be used as a diagnostic test, it should be sensitive and specific**

ACS, HF Biomarkers & Shareholders

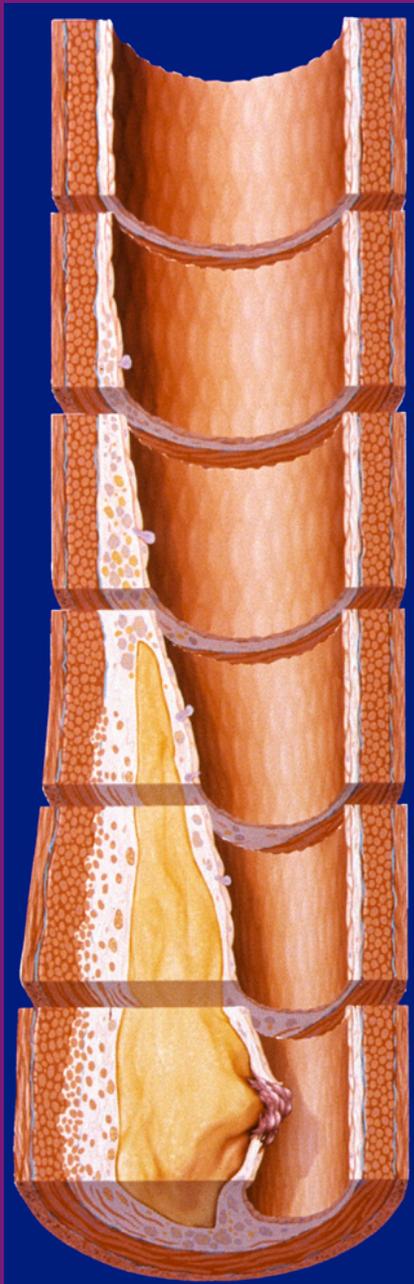
Hospital Based

- **Laboratory Medicine**
 - NACB, IFCC, AACC
- **Cardiology**
 - ESC, AHA, ACC
- **Emergency Medicine**
- **Epidemiology**

Non Hospital Based

- **Diagnostic Companies**
- **FDA, regulatory agencies**
- **Pharmaceutical**
- **Clinical Trials groups**
- **NIH**

Biomarkers: Triage, Diagnostics and Risk Stratification



- Proinflammatory Cytokines

- IL-6, TNF α

- Plaque Destabilization

- MPO, MMPs

- Plaque Rupture

- sCD40L, PIGF, PAPP-A

- Acute Phase Reactants

- hs-CRP

- Ischemia

- IMA, Choline, FFAu

- Necrosis

- cTnT
- cTnI

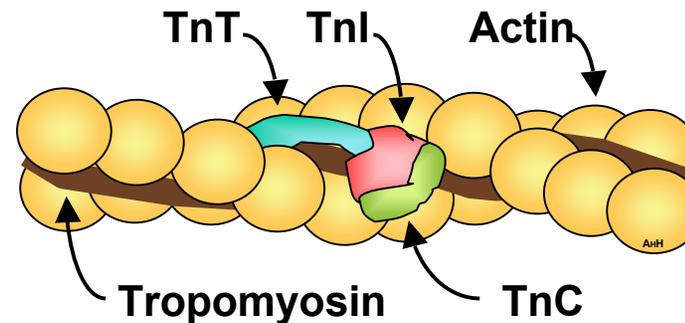
- Myocardial Dysfunction

- BNP
- NT-proBNP

Tomorrow?

Today

Troponin



- Globular protein present in the thin myofilaments
 - Involved in regulation of muscle contraction
- Different isoforms present in skeletal and cardiac muscle
 - Troponin C - binds Ca^{++}
 - Identical in heart and skeletal muscle
 - Troponin I – in absence of Ca^{++} binds to actin, inhibits actinomyosin ATPase induced contraction
 - Cardiac and skeletal muscle isoforms
 - Troponin T- links troponin complex (C, I & T) to tropomyosin
 - Cardiac and skeletal muscle isoforms

Clinical Case

59 yo male is awakened at 0300h with pain in his chest and left shoulder. PMHx significant for 3 prior MI (most recent, 1988) and prior 4V CABG (1988). He presents to the ED at 0430h, and is initially treated with nitroglycerine and aspirin.

0430h: EKG, CK, CK MB, cTnl: All normal

0700h: EKG minor changes

0800h: Coronary angiography: 95% diagonal stenosis. LVEF = 40%.

1030h: Stenting of diagonal

1200h: CK normal, CKMB & cTnl “minimally elevated” on 2nd & 3rd samples



Diagnosis: “Mild heart attack”

- **“...any amount of myocardial damage, as detected by cardiac troponins, implies an impaired clinical outcome for the patient.”**

Biomarker Guideline Efforts

- **National Academy Clinical Biochemistry (draft 2006)**
 - Clinical and analytical
 - Cardiac troponin and natriuretic peptides (BNP)
 - Point of Care (POC)
- **International Federation Clinical Chemistry (2006)**
 - Clinical and analytical - quality specifications
 - Cardiac troponin and natriuretic peptides (BNP)
 - Future biomarkers (2005)
- **ESC/ACC/WHO Global Task Force (2000, draft 2006)**
 - Clinical (and analytical)
 - Cardiac troponin
- **AHA collaboration Soc Chest Pain Centers (2005)**
 - Clinical: unstable angina/NSTEMI
 - Cardiac troponin and CKMB

It's Time For A Change To Troponin ESC/ACC Redefinition for MI

- ◆ **Cardiac troponins are the preferred markers for diagnosis of myocardial injury; increases are not synonymous with ischemic mechanism**
- ◆ **“Myocardial Infarction”- evidence of myocardial damage (increased troponin) in clinical setting of ischemia**
 - **Sampling at admission, 3-6h, 6-9h, 12h**
- ◆ **Prognosis/risk of cardiac events is related in part to the extent of increases of troponin in patients with ischemic mechanism of injury**

ESC/ACC/AHA - Circulation 2000; JACC 2000, EHJ 2000
2005 Global Task Force – Revised Update
2005 NACB – Revised Update

Criteria: Biochemical Markers for Detecting Myocardial Necrosis

- **Increased cardiac troponin defined as measurement $> 99^{\text{th}}$ percentile of reference group**
 - **Maximum concentration of cTnI or cTnT exceeding the decision limit (99^{th} percentile for reference control group) at least once during initial 24h after index event**
- **Goal for imprecision ($\%CV$) at 99^{th} percentile should be $\leq 10\%$**

ESC/ACC/AHA - Circulation 2000; JACC 2000, EHJ 2000
2005 Global Task Force – Revised Update
2005 NACB – Revised Update

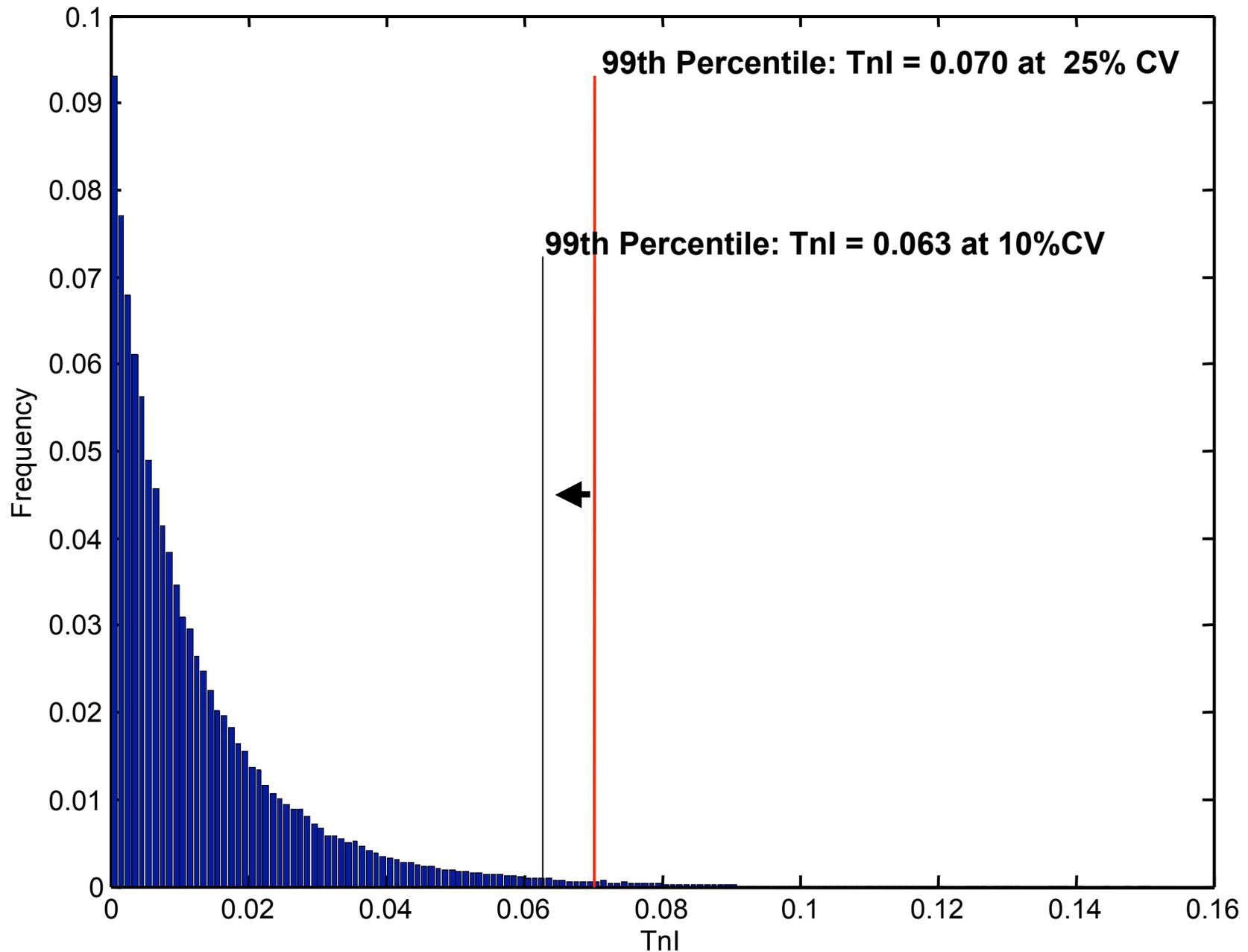
Reference Limit Study: Cardiac Troponin

<i>99th Percentiles</i>	N	Dimension	Elecsys cTnT		Access	
<i>Manufacturer 99th</i>		<i>0.07</i>	<i>0.01</i>		<i>0.03</i>	
ALL SUBJECTS	671	0.06	<0.010		0.08	
Males	300	0.06	<0.010		0.10	
Females	371	0.04	<0.010		0.04	
<i>p-value</i>		<i>0.6</i>	<i>0.3</i>		<i>0.045</i>	
Caucasians	402	0.04	<0.010		0.07	
African American	196	0.07	<0.010		0.12	
		NS	NS		NS	

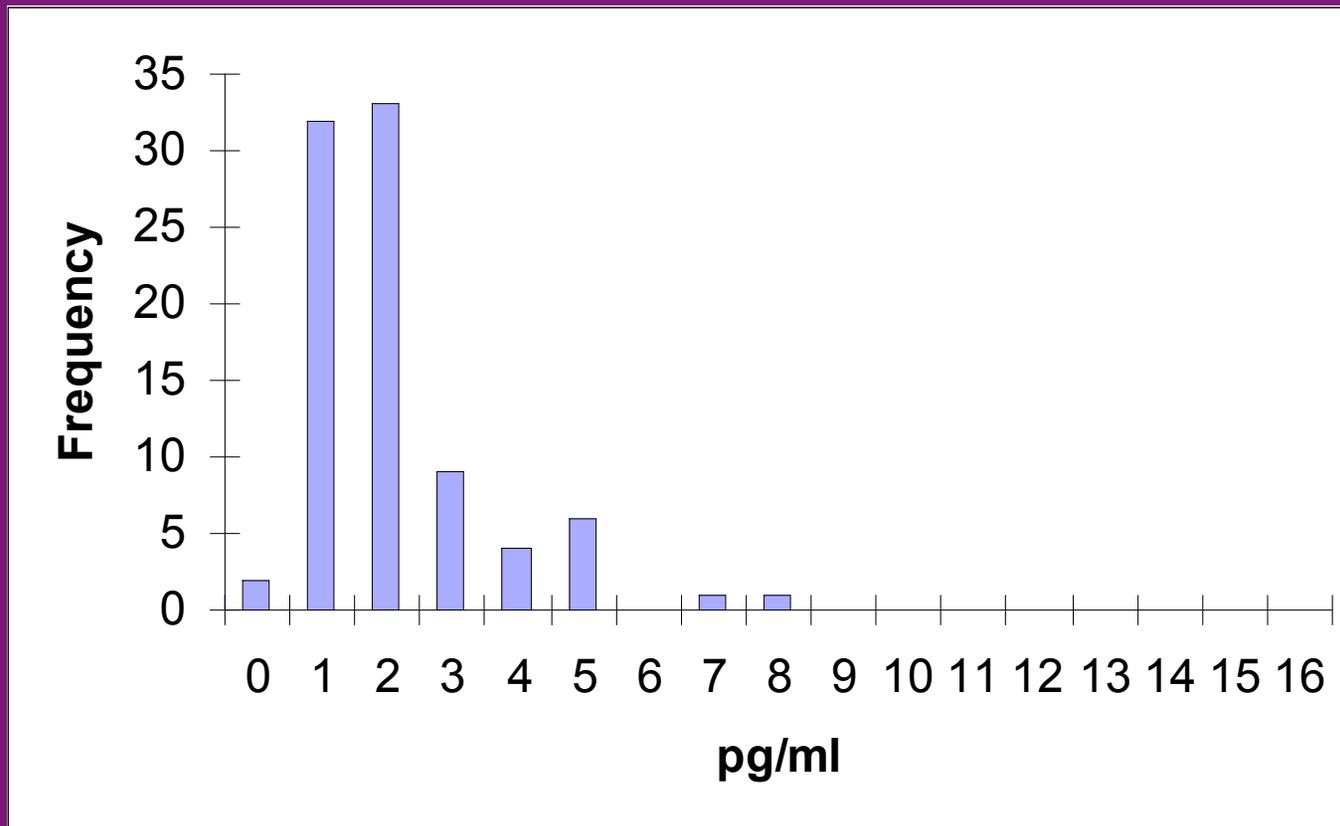
Plasma 99th Percentile Reference Cutoffs – Cardiac Troponin 2005 Study

	N	Dimensin	Elecsys	Access	Architect	Vitros ES
Manufacturer		0.07	<0.01	0.03	0.012 (n=224)	-
Studies						
Subjects	671	0.06	<0.01			
Males	300	0.06	<0.01			
Females	371	0.04	<0.01			
Subjects	2992			0.04	0.025	0.037

Determining Reference Ranges: Assay Dependent Validation



Singulex



Reference Limit Study: CKMB mass

99th Percentiles	N	Dimension	Elecsys	Vitros	Access
<i>Manufacturer 99th</i>					
Manufacturer 97.5th			F 2.8 / M 4.9	3.4	3.8
Manufacturer 95th		3.6			4.0
ALL SUBJECTS	671	3.8	6.2	4.10	6.8
Males	300	5.1	7.6	4.60	9.3
Females	371	3.1	4.1	2.90	4.4
<i>p-value</i>		<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>	<i>0.0001</i>
Caucasians	402	3.2	6.1	4.10	6.6
African American	196	6.0	7.2	4.60	11.0
Tosoh 5.7 / 3.0					
<i>p 0.0001</i>					

Objectives - Quality Specifications of Biomarker Assays

- **Manufacturers endorse & consistently follow**
- **Package inserts to include uniform information**
- **Publication in peer-reviewed journals**
- **Encourage regulatory agencies to adopt a minimal and uniform set of criteria for manufacturers to provide when seeking clearance of assays**

Quality Specifications • Troponin Assays

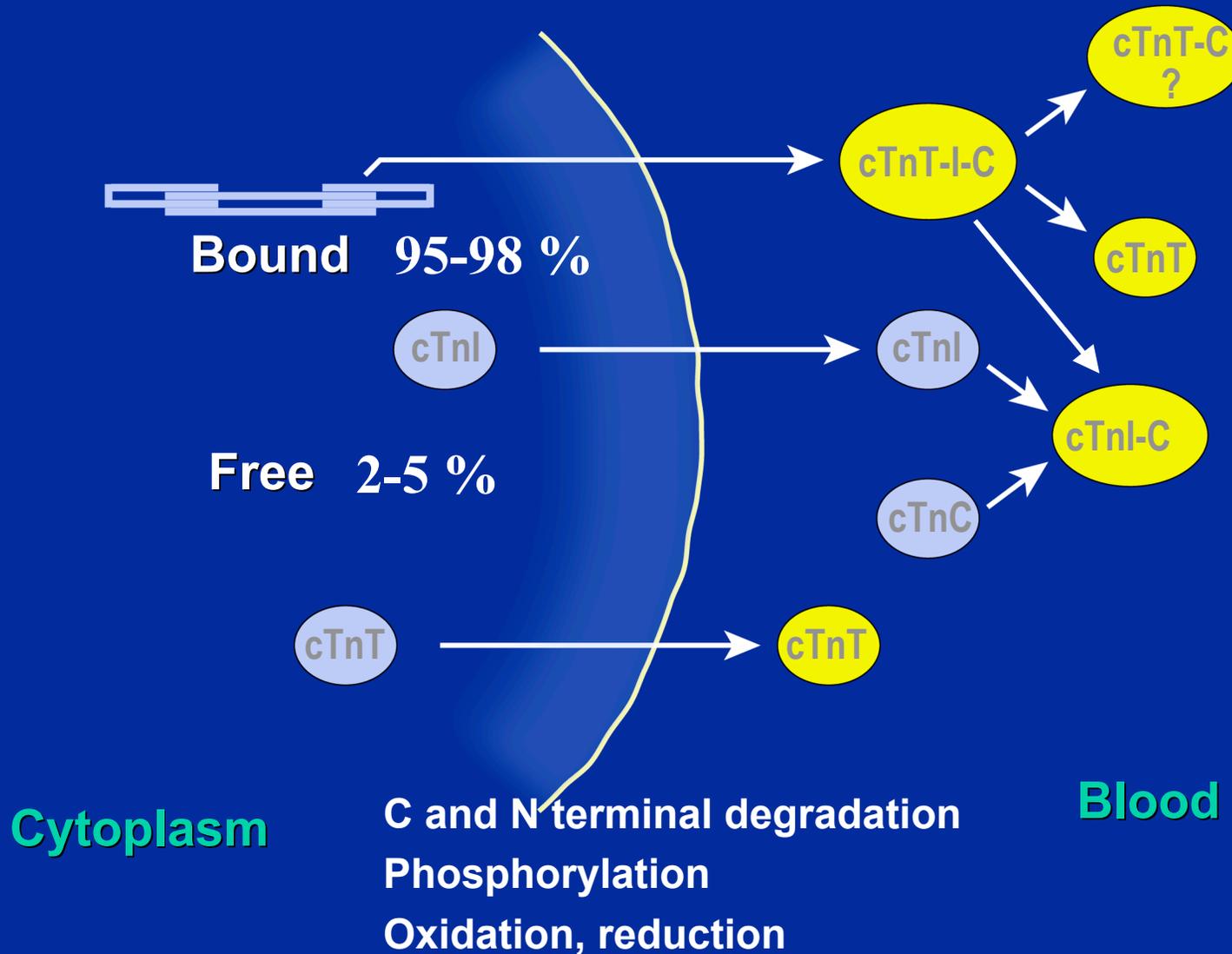
◆ Analytical Factors

- Antibody specificity – recognize epitopes in stable part of molecule and equimolar for all forms
- Influence of anticoagulants
- Calibrate against natural form of molecule
- Define type of material useful for dilutions
- Demonstrate recovery and linearity of method
- Describe detection limit and imprecision (10% CV)
- Address interferents, i.e. RF, heterophile antibodies

◆ Pre-analytical Factors

- Storage time and temperature conditions
- Centrifugation effects – gel separators
- Serum – plasma – WB correlations

Postulate for Troponin Release



Degradation of Cardiac Troponin I in Human Necrotic Heart Tissue and Serum (Clin Chem 1998)

Antibody Heterogeneity and Standardization Issues

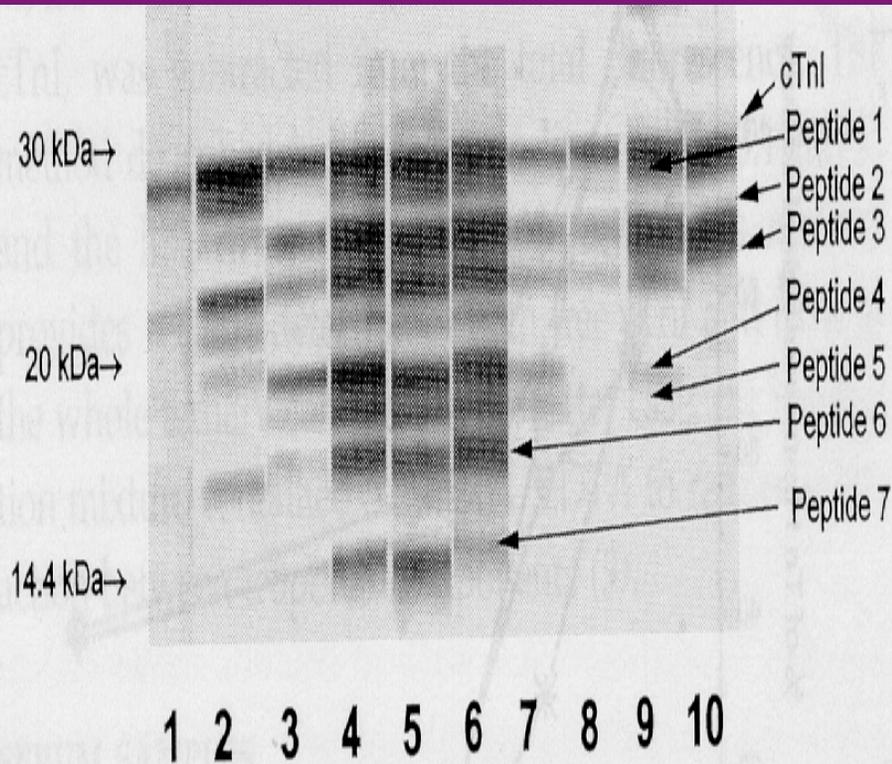


Fig. 2. Recognition of endogenous proteolytic fragments of cTnI by different MAbs.

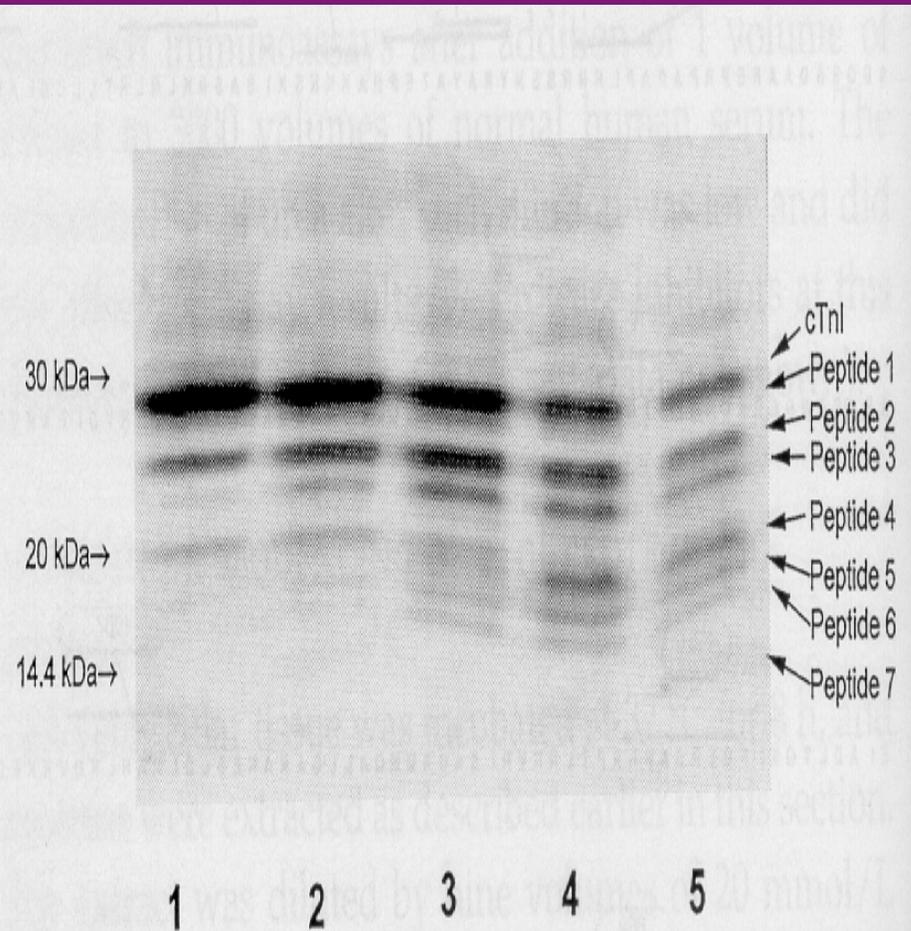
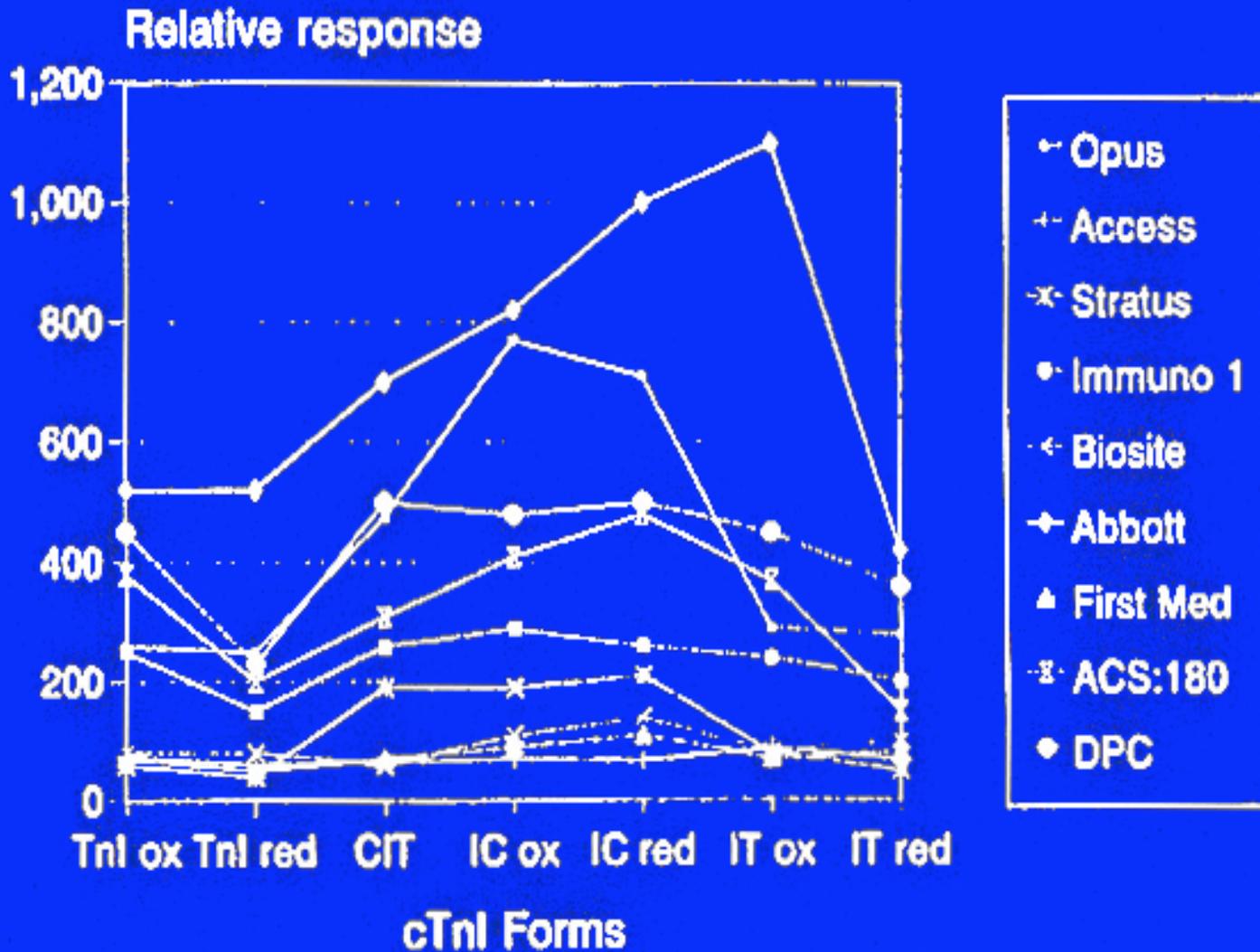


Fig. 3. Kinetics of cTnI endogenous proteolysis in heart tissue.

Correlation of cTnI assays



Clin Chem 1999

Overview of Cardiac Troponin Assays

<u>Assay</u>	<u>LLD</u>	<u>99th Percentile</u>	<u>WHO-ROC Cutoff</u>	<u>10%* CV</u>
Abbott ARCH	0.009	0.012	0.3	0.032
AxSYM ADV	0.02	0.04	0.4	0.16
**i-STAT	0.02	0.08 (WB)	ND	0.1
Bayer Centaur	0.02	0.1	1.0	0.35
Ultra	0.006	0.04	0.9	0.03
Beckman Accu	0.01	0.04	0.5	0.06
**Biosite Triage	0.05	<0.05	0.4	NA
bioMerieux Vds	0.001	0.01	0.16	0.11
Dade RxL	0.04	0.07	0.6-1.5	0.14
**CS	0.03	0.07	0.6-1.5	0.06
DPC Immulite	0.1	0.2	1.0	0.6
MKI Pathfast	0.006	0.01	0.06	0.06
Ortho Vitros	0.02	0.08	0.4	0.12
ES (R&D)	0.012	0.032	0.12	0.053
**Response	0.03	< 0.03 (WB)	ND	0.21
Roche Elecsys	0.01	<0.01	0.03	0.03
**Reader	0.05	<0.05 (WB)	0.1	ND
Tosoh AIA	0.06	0.06	0.31-0.64	0.06

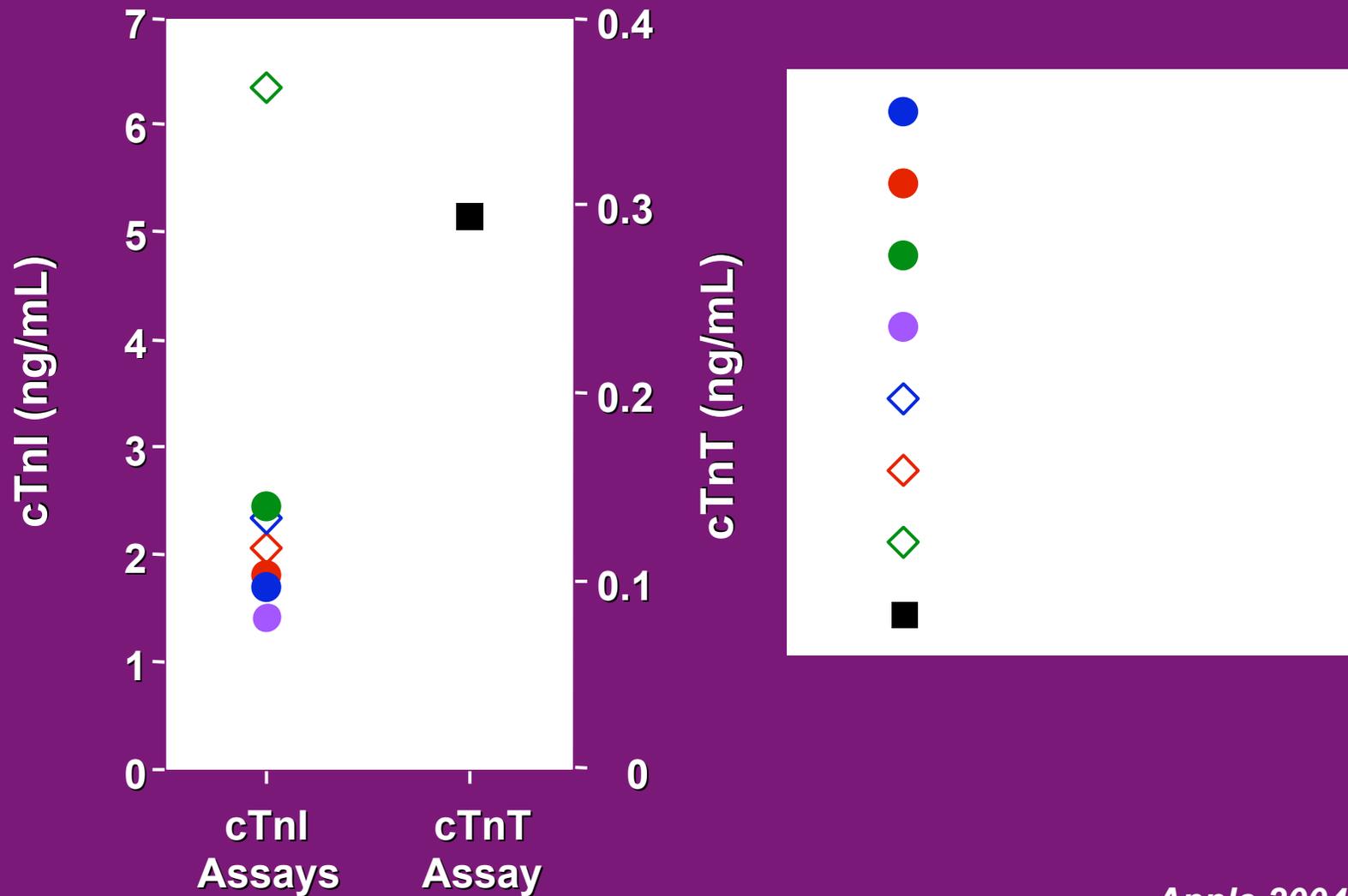
•*Per manufacturer; ** POC Assay

•Adapted - Apple Am Heart J 2002

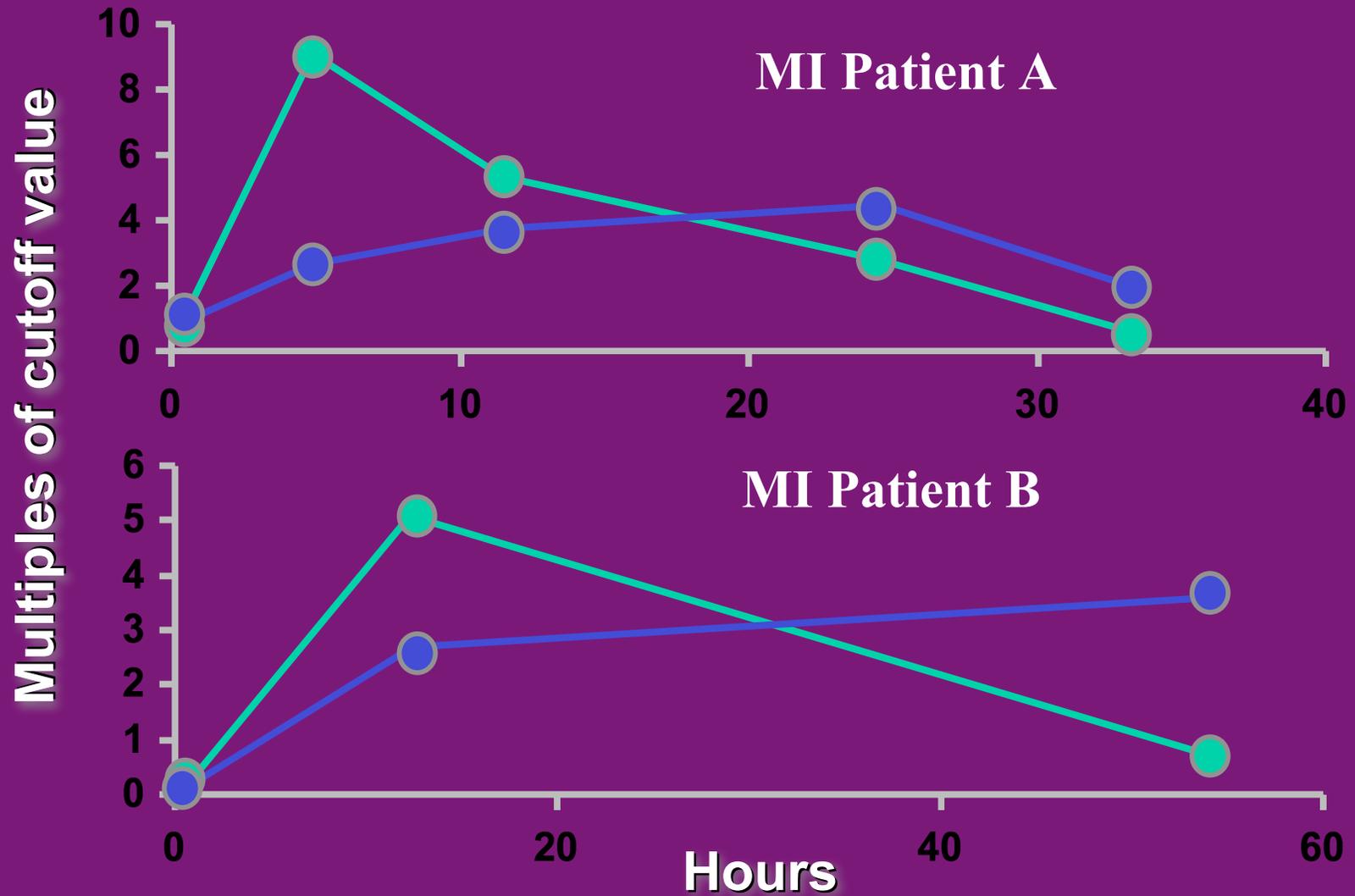
Cardiac Marker Scottish External QA Scheme (EQAS) June 2005

Method	Mean ng/mL	% CV	N
Abbott Architect	0.25	8.5	9
Beckman Accu	0.16	10.5	41
Bayer Centaur	0.45	9.6	38
Dade CS	0.44	8.9	20
Tosoh AIA	0.14	22.0	11
DPC Immulite 2000	0.79	149	9
Roche 3rd gen	0.08	10.4	114

Cardiac Troponin Assay Response to Positive Human LiHep Plasma Specimen



Correlation for Commercial Troponin Assays



Standardization Efforts

- **AACC**
 - **cTnI Standardization Committee**
 - **National Institute Standards & Technology (NIST)**
 - **CKMB mass Standardization Committee - completed**
- **IFCC - Committee on Standardization of Markers of Cardiac Damage (C-SMCD)**
 - **Institute of Reference Materials and Measurements (IRMM)**
 - **Myoglobin in progress**
 - **BNP/NT-proBNP – 2005 (NIST, IRMM)**

Standardizing Assays Impacts Clinical Practice

Computer model of
complex crystal structure
of human protein cardiac
troponin (Graphic Courtesy
Protein Data Bank)



National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 2921

Human Cardiac Troponin Complex

This Standard Reference Material (SRM) is primarily intended for use in evaluating the accuracy of clinical procedures for the determination of cardiac troponin I (cTnI) in human serum. It can be used to validate working or secondary reference material. A unit of SRM 2921 consists of five vials, each containing approximately 115 µL of a dilute solution of human cardiac troponin complex.

The troponin complex was purified from human heart tissue and consists of three subunits: troponin T (cTnT), troponin I (cTnI), and troponin C (cTnC). The choice of a human cardiac troponin complex was made with the assistance of the Cardiac Troponin I Standardization Subcommittee of the American Association for Clinical Chemistry (AACC). Through two interlaboratory comparison studies, various troponin preparations, including recombinant and native troponin complexes of the troponin T, troponin I, and troponin C subunits, recombinant troponin I/troponin C complex, and recombinant and native cTnI, were evaluated using fifteen commercial cTnI assays [1]. Based on the results of these interlaboratory comparison studies, the human cardiac troponin complex was chosen for its ability to harmonize clinical assays and for its commutability.

Certified Human Cardiac Troponin Concentration. The certified concentration value of human cTnI was determined through a combination of amino acid analysis and liquid chromatography (LC). The amino acid analysis used SRM 2389 Amino Acids in 0.1 mol/L Hydrochloric Acid as an external standard.

Certified cTnI Concentration: 31.2 mg/L ± 1.4 mg/L

The certified value is the weighted mean of results obtained from two methods. The uncertainty in the certified concentration is calculated as $U = k u_c$. The quantity u_c is the combined standard uncertainty calculated according to the ISO/NIST Guide [2], where u_c is intended to represent, at the level of one standard deviation, the combined effect of within-method variation for both methods and between method variation for the two methods. The coverage factor, k , is determined from the Student's t -distribution corresponding to the appropriate associated degrees of freedom and a 95% level of confidence.

Reference Concentration Values: Reference concentration values for cTnI and cTnC are provided in Table 1. The reference values are noncertified values that are the best estimates of the true values, however, these values do not meet NIST criteria for certification and are provided with associated uncertainties that may reflect only measurement precision, may not include all sources of uncertainty, or may reflect a lack of sufficient statistical agreement among multiple analytical methods.

Expiration of Certification: The certification of this SRM is valid until 30 January 2010, within the measurement uncertainties specified, provided the SRM is handled and stored in accordance with the instructions given in this certificate. However, the certification is invalid if the SRM is contaminated or modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Coordination of the technical measurements leading to certification of this SRM was performed by D. M. Bunk and M. J. Welch of the NIST Analytical Chemistry Division.

Wille E. May, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 30 April 2004

Robert L. Walters, Jr., Acting Chief
Measurement Services Division

SRM 2921

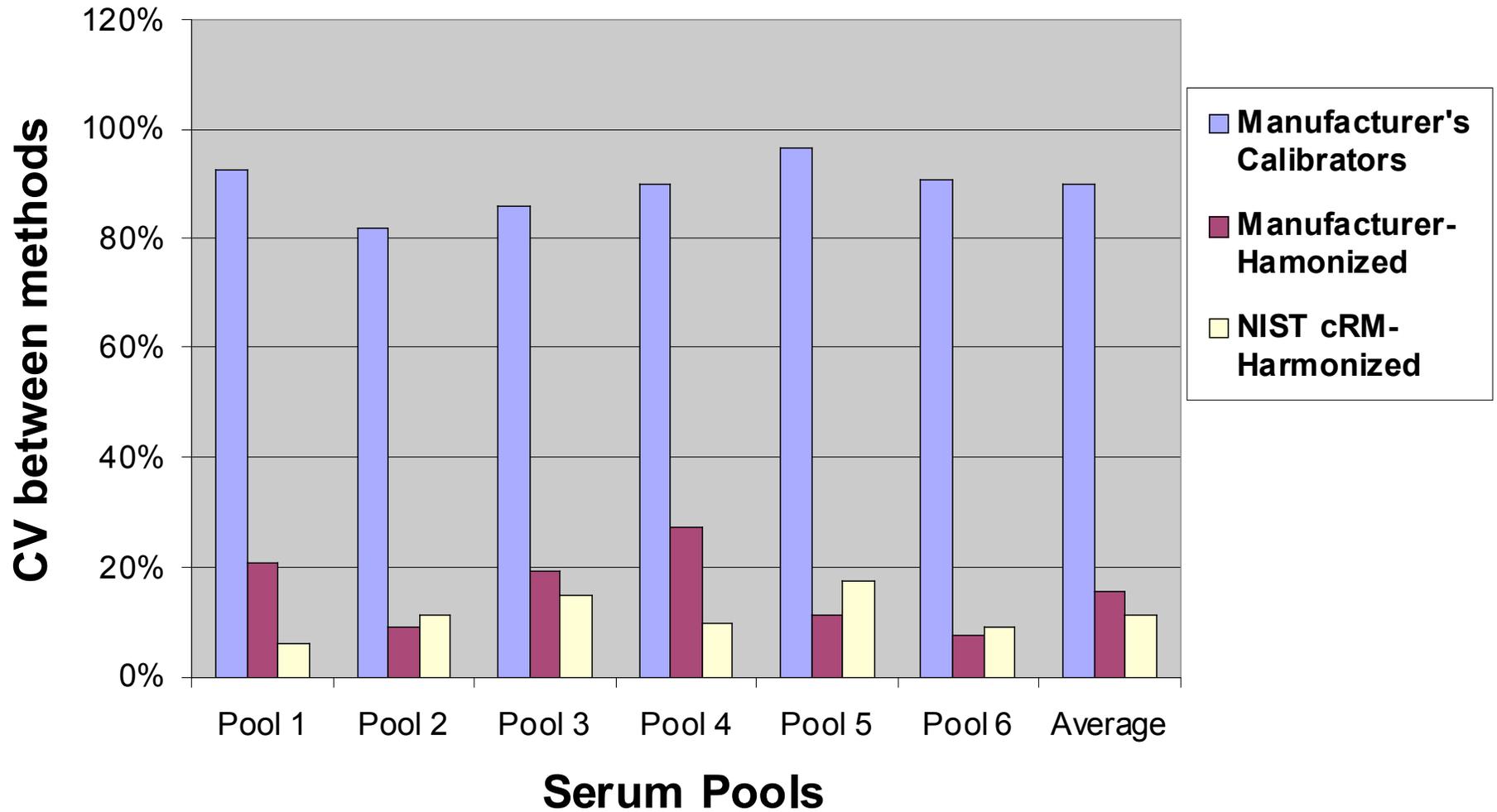
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**SRM 2921
for cTnI
Cooperative
effort
between
NIST, AACC
and IVD**

www.nist.gov



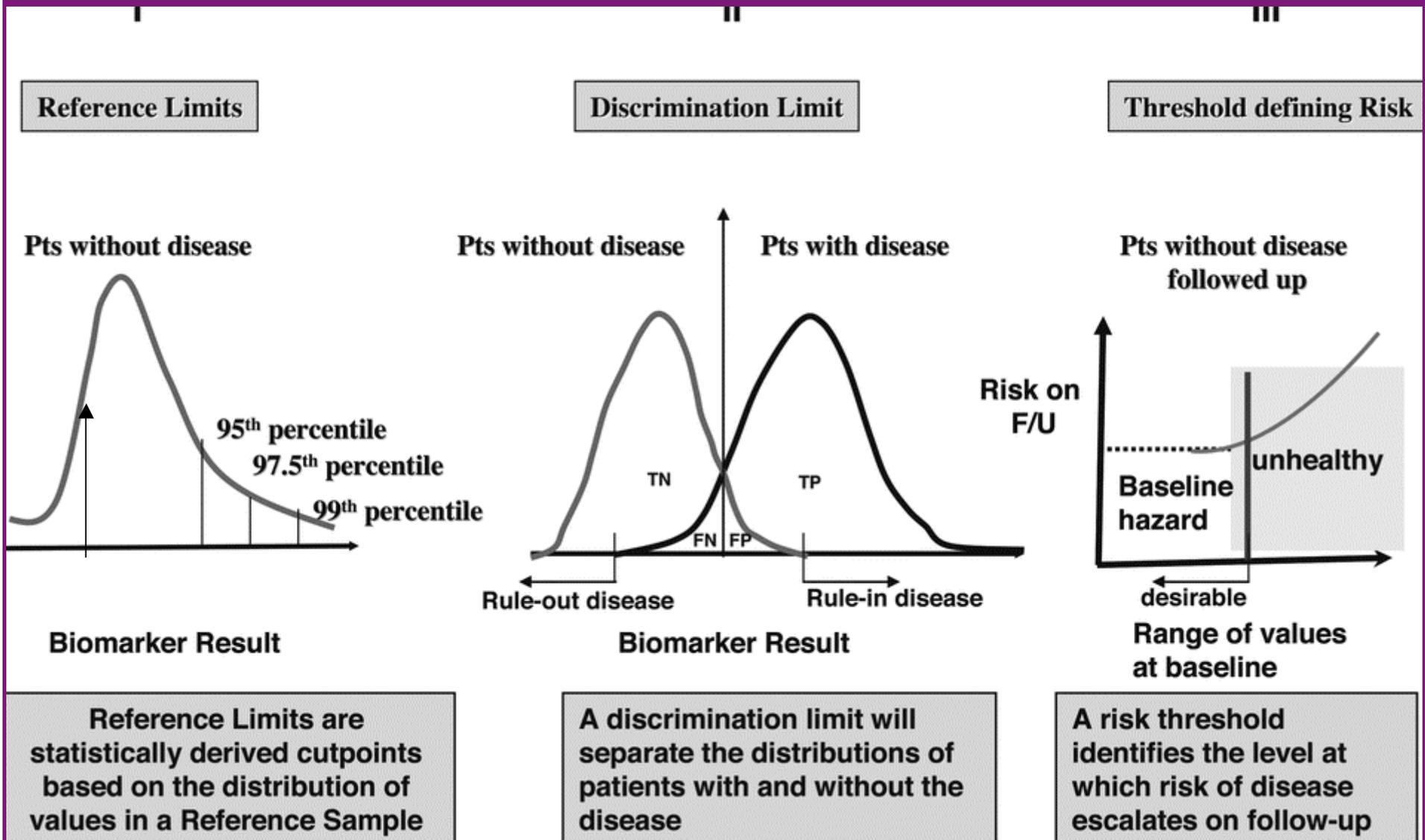
cTnI Harmonization



AACC cTnI Standardization Committee; Christenson et al. Clin Chem 2006

Impact of Medical Decision Cutoffs

Circulation 2006



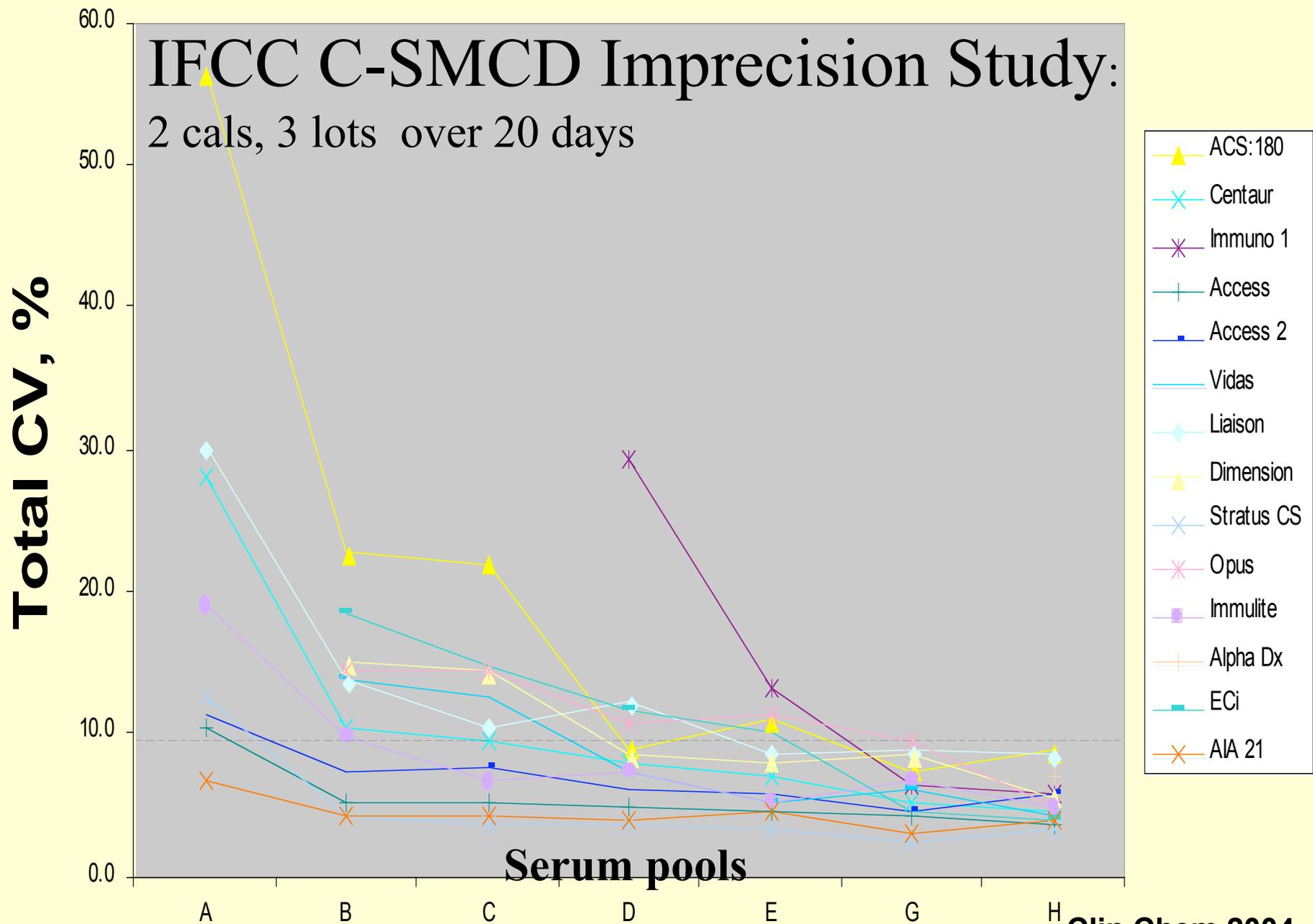
Diagnostic Implications in 1719 Consecutive ACS Admissions Based On Cutoffs

Cutoff Conc.	cTnI + MB -	cTnI + MB +	cTnI - MB -	cTnI - MB +
ROC	4.2% (n=73)	9.2%	78.4%	8.1%
10% CV	5.7% (↑ 34%) (n=98)	11.3%	72.6%	10.4%
99 th	12.2% (↑ 186%) (n=209)	12.9%	66.1%	8.8%

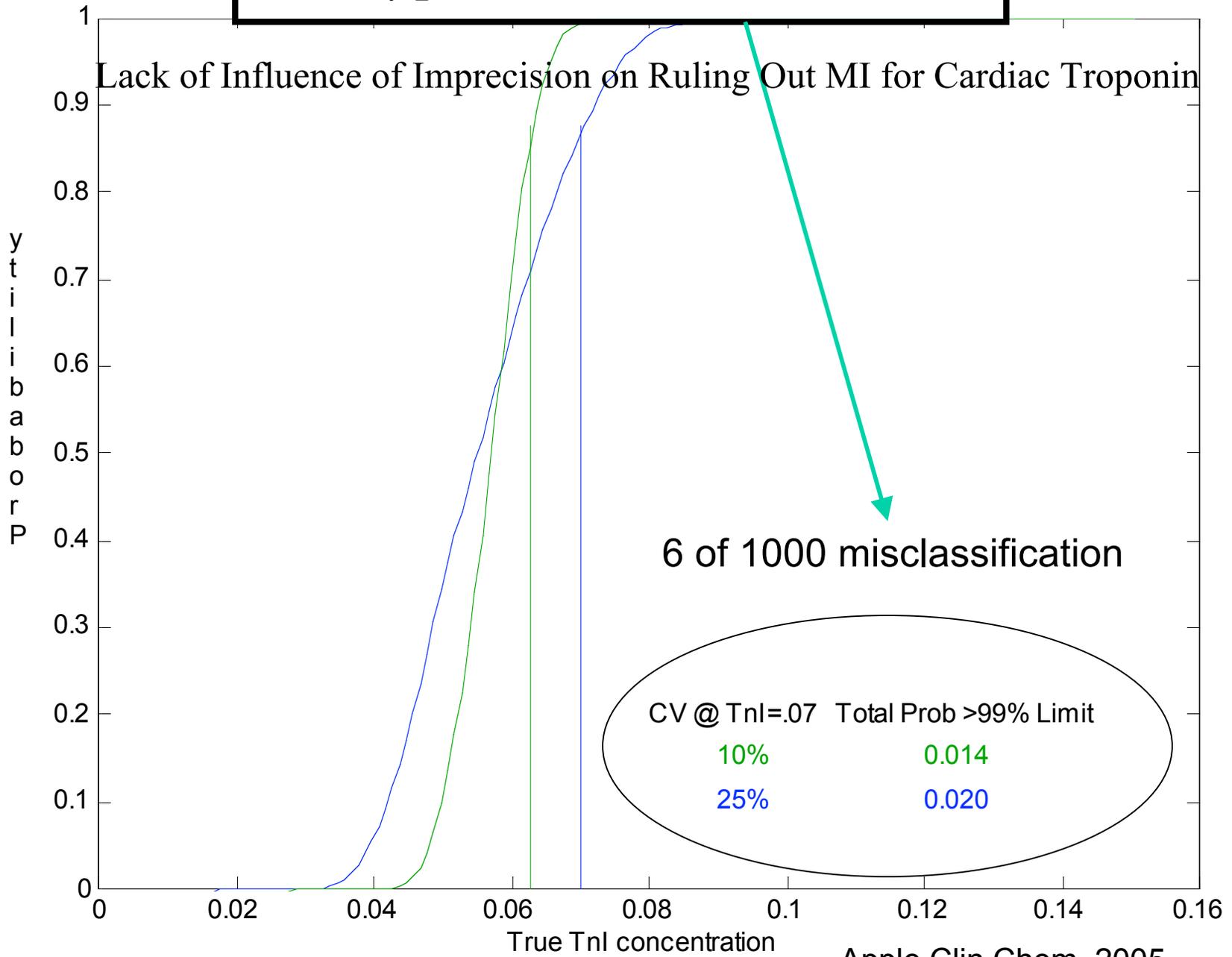
cTnI-, MB + = false positives

Dade Dimension: ROC 0.6, 10%CV 0.4, 99th 0.1 ng/mL

IFCC C-SMCD Imprecision Study: 2 calcs, 3 lots over 20 days



Probability ≥ 1 out of 3 Results Exceed 99th Percentile Limit



Cardiac Troponin (cTn) T or I as Bridging Biomarkers

- Animal models with induced cardiac muscle injury show concordance with human cTn response in:
 - Cardiac tissue specificity
 - Onset, magnitude and duration of cTn response
 - Associated cardiac histologic changes
- Commercial cTnI immunoassays - variety of proprietary antibody reagents, cTnT assays - uniform antibody reagents, All: non-standardized reference material



The ILSI-HESI Biomarkers Committee, Cardiac (Troponins) EWG Initiative

- **Major Goals:**
- Analytical validation of selected cTn assays, establishing degree of immunoreactivity, linearity, detection limit and imprecision of assays:
 - Rat, Beagle Dog, Cynomologus and Rhesus monkey
- Biological Validation to establish diagnostic window for serum cTn with drug-induced cardiomyocyte injury and examine the:
 - correlation with cardiac histopathology
 - diagnostic advantage to measuring cTnI +/-or cTnT
 - clearance rate of cTn in laboratory species



Acknowledgements

Participating Members of The Troponin Working Group

ILSI HESI Scientific Program Manager

Syril Pettit, Michael Holsapple

Clinical Collaborators

Fred Apple, MaryAnn Murakami

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Brian Berridge, Eric Schultze, Lou Mylecraine,

Mike Stonebrook, Martin Lamb, Denise Bounous,

Nigel Roome, Calvert Loudon, Gareth Evans, Malcolm York



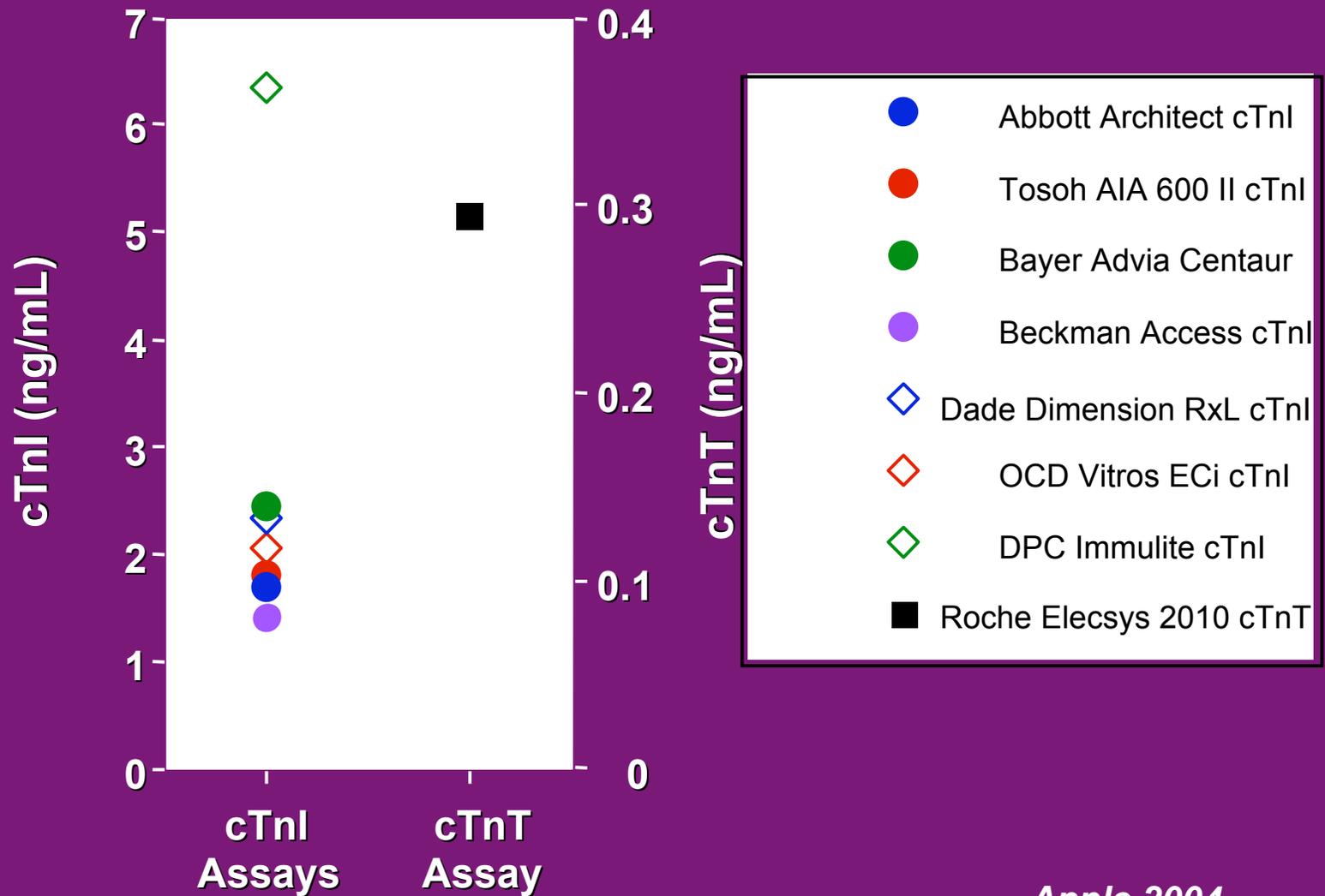
H E S I

ILSI-HESI Troponins Subcommittee: Analytical Validation of cTn Assays

- High cTn serum generated for each of the test species
 - rat, dog, monkey
 - Isoproterenol, norepinephrine or coronary artery ligation
- High, medium and low Tn(+) linear dilutions, and negative serum pools evaluated on:
 - Abbott Architect cTnI
 - Bayer Centaur cTnI
 - Beckman Access cTnI
 - DPC Immulite cTnI
 - Dade Dimension RxL cTnI
 - Ortho-Clinical Diagnostics Vitros ECI cTnI
 - Roche Elecsys 2010 cTnT
 - Tosoh AIA 600 II cTnI
 - Species-specific TnI EIA [Life Diagnostics]



Cardiac Troponin Assay Response to Positive Human LiHep Plasma Specimen



Apple 2004

FDA Cleared Cardiac Troponin Assays

<u>cTn Assay</u>	<u>LLD</u> <u>(ng/mL)</u>	<u>99th</u> <u>Percentile</u>	<u>10% CV</u>
Abbott Arch	0.008	0.017	0.032
Bayer Centaur	0.02	0.07	0.35
Beckman Access	0.01	0.04	0.06
Dade RxL	0.04	0.07	0.14
DPC Immulite	0.2	0.2	0.6
Ortho Vitros	0.02	0.08	0.12
Roche Elecsys	0.01	0.01	0.03
Tosoh AIA	0.06	<0.06	0.06

Species EIA

Dog	0.156*
Monkey	0.156*
Rat	1.56*

* lowest standard

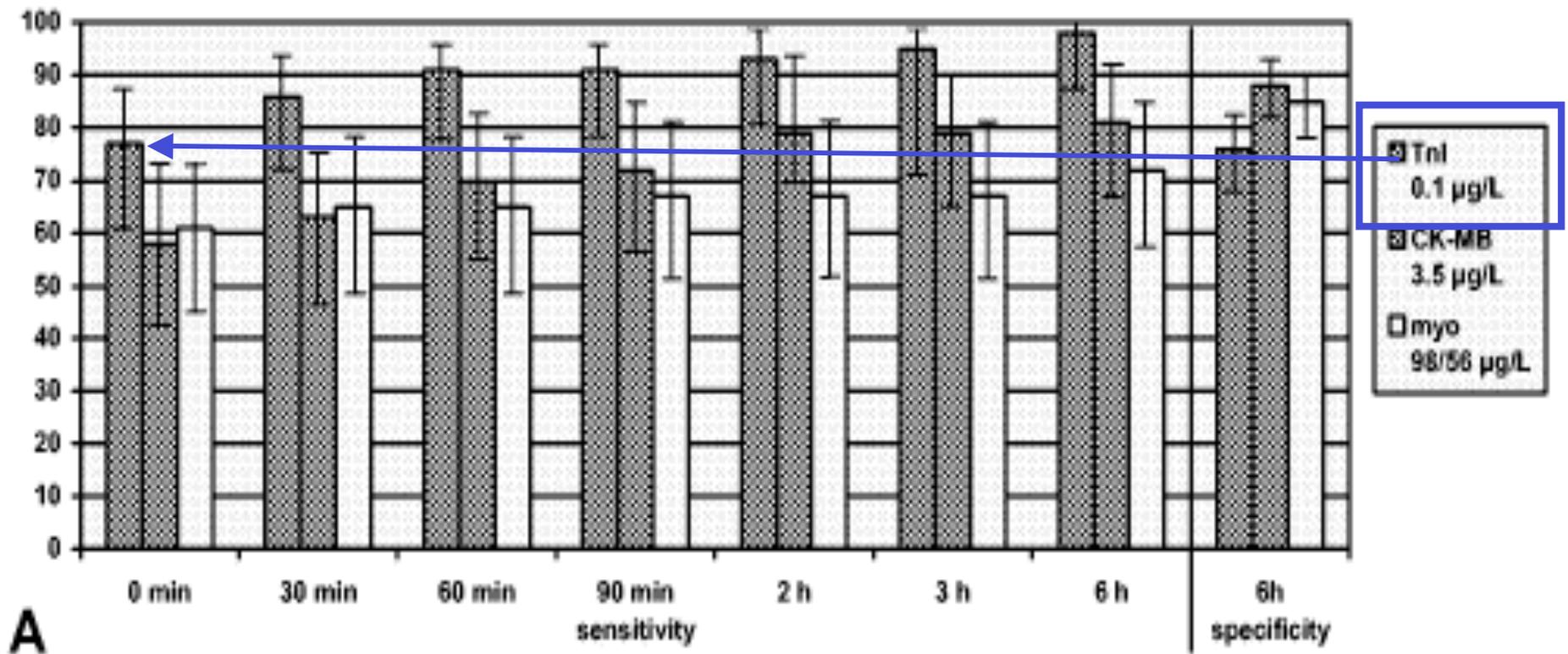
Apple 2004

Analytical Validation Phase 1B

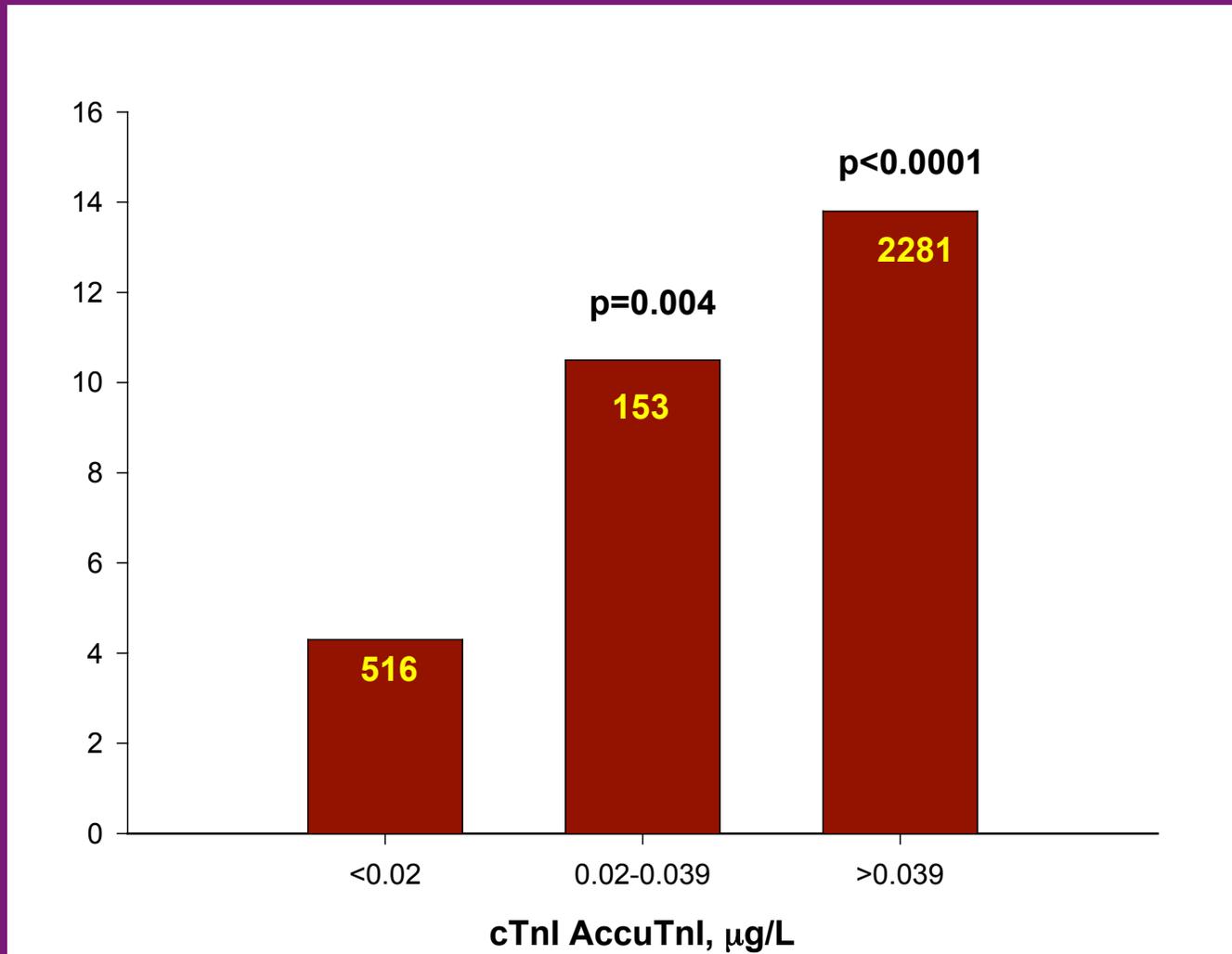
- **To evaluate the imprecision of 7 cardiac troponin assays for 4 animal species/strains**
- **Negative & high troponin serum pools for each species supplied by HESI Troponin Working Group**
- **Targeted Troponin serum pools (A-E) created over approximate range 0.8, 0.4, 0.2, 0.1, 0.05 ng/mL, prepared using Dade Dimension RXL cTnI assay**
- **Each pool analyzed 10 times (in duplicate) over 10 working days**
- **Pools were created for each species to span the critical range of the assay**



Detection of MI: cTnI vs. CK-MB & Myoglobin Utilizing 99th Percentile Cutoff Concentration



Prediction of Death/AMI by TnI in ACS



Summary - Cardiac Troponin

- **Troponin-omics- cardiac troponin is here to stay...**
- **Second and third generation cTnI and cTnT assays are analytically and clinically acceptable**
 - **Detection of myocardial injury**
 - **Aid in diagnosis of myocardial infarction**
 - **Risk stratification**
- **We need to do a better job at defining “what is normal”**
- **Preanalytical & analytical issues continue to perplex assays**
 - **Challenge the reliability of potential new biomarkers**
- **Educational struggle for all users: industry, pharma, clinicians and laboratorians to understand use of troponin**
 - **KNOW YOUR ASSAY**